RNA Amplification of Dorsal Root Ganglion Neurons Retrogradely Labeled with Di-i Signaling Chronic Pain and Fatigue


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INTRODUCTION

- In muscles, there are neurons of groups III and IV that correspond to two kinds of afferents or nerves.
- In previous studies regarding the responses of these afferents in skeletal muscle, single mechanical stimuli evoked responses.
- Chemical stimuli are equally important, as they are responsible for the pain and fatigue states during and after mechanical stimuli.
- Unlike previous studies involving analysis of single metabolites, cultured dorsal root ganglion (DRG) neurons are exposed to combinations of the chemical metabolites responsible for pain and fatigue, including lactic acid, ATP, and protons (altered pH).
- Key receptors of these metabolites were found to be ASICs, P2X, and TRPV (Light et al., 2008).
- By using a combination of the metabolites, which are agonists for these receptors, the responses are enhanced, viewed, and analyzed by calcium imaging showing that the metabolites are crucial in signaling pain and fatigue in skeletal muscle (Light et al., 2008).
- Carrageenan injections causes inflammation which evokes similar response as pain and fatigue.

PURPOSE

A protocol is being developed to amplify the RNA from the small amount of Di-I labeled sensory DRG neuron cells in order for the analysis of calcium imaging to be successful. The protocol will include TransPlex® Whole Transcriptome Amplification Kit from Sigma® with JumpStart™ Taq DNA Polymerase, to determine its effectiveness in RNA amplification.

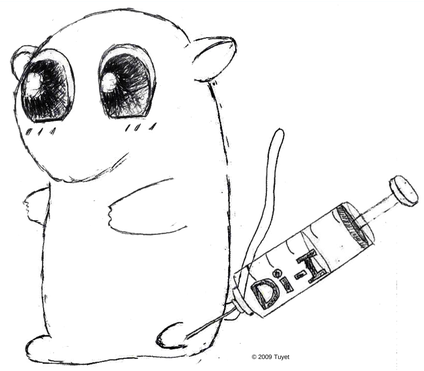
HYPOTHESIS

With successful use of the amplification kit, the Di-I labeled cells will be amplified approximately 1000-10,000 fold, from nanogram quantities to micrograms of RNA.

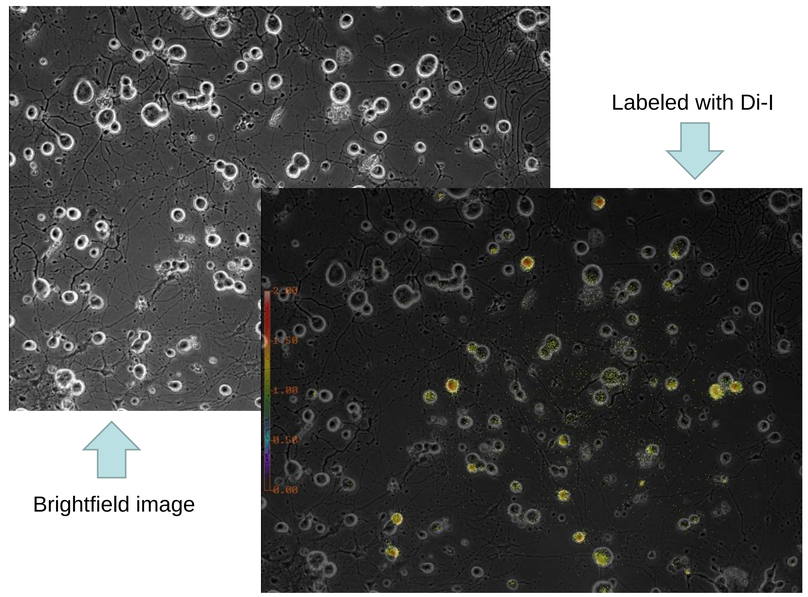
METHODS

Normally, RNA from whole DRGs is extracted and converted into a cDNA library by using ABI's Multiscribe reverse-transcriptase, which is then analyzed using real-time quantitative PCR. Due to the small number of individual Di-I labeled cells harvested in this procedure, the yield of cDNA is too low to perform meaningful real-time quantitative PCR. Thus, by using TransPlex® Whole Transcriptome Amplification Kit from Sigma® with JumpStart™ Taq DNA Polymerase, there should be a substantial yield of amplicfied RNA.

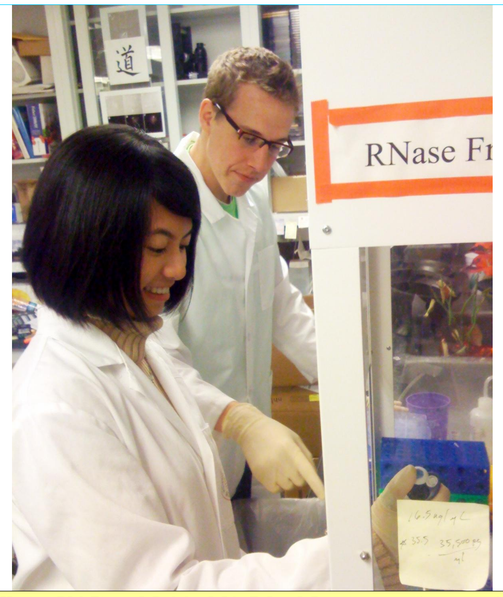
1 **Injection of a florescent dye called Di-I into the lower leg muscles of nine day old mice a week before the procedure allows the sensory DRG neurons to fluoresce under the microscope. 24 hours before dissection carrageenan is injected into the muscles.**



2 **Using the Di-I fluorescence to identify individual retrogradely labeled cells from muscle, small numbers of labeled cells are collected and quick-frozen.**

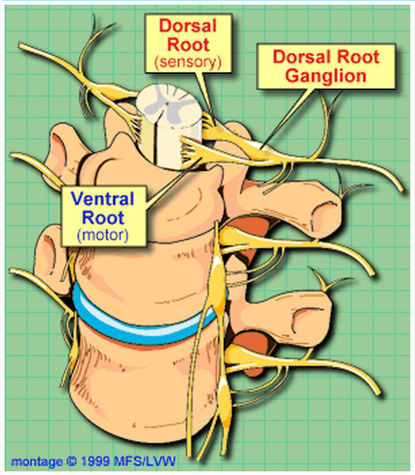


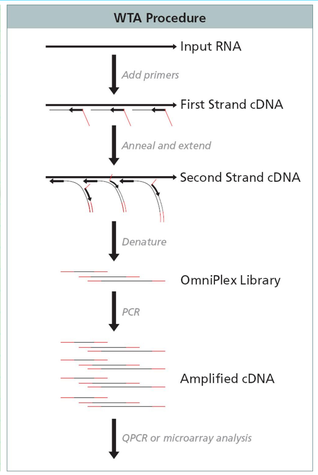
3 **Library synthesis: Sample RNA is reverse transcribed with non-self-complementary primers with a quasi-random 3' end and a universal 5' end.**



References: **Light AR, Hughen RW, Zhang J, Rainier J, Liu Z, Lee J.** Dorsal root ganglion neurons innervating skeletal muscle respond to physiological combinations of protons, ATP, and lactate mediated by ASIC, P2X, and TRPV1. *J Neurophysiol.* 100:1184-201(2008).

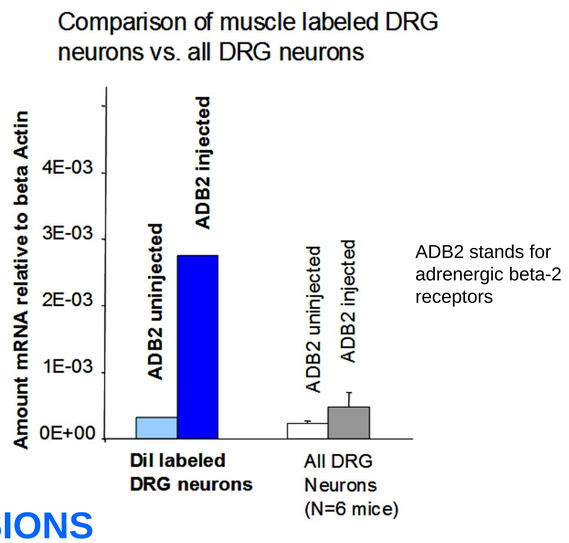
4 **Library synthesis: As polymerization proceeds, displaced single strands serve as new templates for primer annealing and extension.**





5 **Library amplification: The resultant OmniPlex® cDNA library is amplified by PCR with the universal primer to produce Whole Transcriptome Amplification (WTA) product.**

RESULTS



CONCLUSIONS

1) According to the results, there is a large increase in the detectable amount of adrenergic beta-2 receptors when the mouse is injected with both carrageenan and labeled with Di-I; as opposed to DRG neurons with no Di-I injection, which yields less than a fourth as much.
2) Although the first results appear promising ,the RNA amplification study is still in the process and its effectiveness is being determined.
3) The implications of this research is that if there are accurately labeled muscle DRG neurons, the results will produce meaningful real-time PCR products to be used in the future.